Sudan University of Science and Technology (SUST)

College of Graduate Studies

Estradiol and calcium levels among Female patients with Polycystic Ovarian Syndrome in Khartoum State

مستوى الاستراديول والكالسيوم لدى الاناث المصابات بمتلازمة التكيس المبيضي المتعدد في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirement of M.Sc. degree in medical laboratory science -Clinical Chemistry

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الآية الكريمة

قال تعالى :

(يأيها الذين آمنوا إذا قيل لكم تفسحوا في المجالس فأفسحوا يفسح الله لكم و إذا قيل انشزوا فانشزوا يرفع الله الذين آمنوا منكم والذين أوتوا العلم درجات والله بما تعملون خبير).

المجادلة الآية (11).

Dedication

I dedicate this research for whom that makes my dreams become true

My families.

Special dedicate to .....

My father

My mother

For every one that help me to achieve this research

My friends, My teachers.

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Abstract

Background: Polycystic ovarian syndrome is associated with increased ovarian and adrenal androgen secretions and hyperandrogenism and resultant hirsutism, hyperinsulinemia and central obesity. Although vitamin D primarily plays a role in bone metabolism, it has important functions in the reproductive system. Vitamin D receptors are found in ovarian and endometrial tissues and play an important role in steroidogenesis. Vitamin D deficiency is more frequently encountered in patients with polycystic ovarian syndrome.

Objective: This study was carried out to assess the level of calcium, albumin, AMH and estradiol in female patients with polycystic ovarian syndrome.

Material and Methods: forty newly diagnosed PCOS subjects aged 18-35 years and 40age-matched healthy women as control group at Khartoum state.

Results: The mean level of plasma calcium was significantly decreased (p.value 0.000) whereas the mean level of AMH was significantly increased (p. value0.000) in patients with polycystic ovarian syndrome as compared to control group. On the other hand the mean level of estradiol and albumin were insignificantly decreased (p.value 0.086 and 0.283) respectively. In conclusion polycystic ovarian syndrome decreases the level of calcium, estradiol and increases the level of AMH.

مستخلص الدراسة

مقدمة: تعتبر متلازمة تكيس المبايض مرتبطة بزيادة انتاج المبيض والغدة الفوق الكلوية للاندروجين و فرط الاندروجين و كثافة الشعر و فرط الانسولين والبدانة .

بالرغم من ان فيتامين د يلعب دورا اساسيا في نمو العظام لديه وظائف مهمة ايضا في الجهاز التناسلي ،توجد مستقبلات فيتامين د في الرحم و بطانة الرحم و يلعب دورا أساسيا في عملية انتاج وتوليد الاستيرويد .في كثير من الاحيان واجهت نقصان فيتامين د في مرضى متلازمة تكيس المبايض.

الهدف: أجريت هذه الدراسة لتقييم مستوى الكالسيوم ،الألبيومين ،الهرمون المضادة للميليريان و هرمون الاستراديول في دم النساء اللاتي يعانين من متلازمة تكيس المبايض.

الأساليب والمواد: تم إختيار أربعين من الذين يعانون من متلازمة تكيس المبايض وأربعون أصحاء كمجوعة تحكم للمقارنة وذلك في ولاية الخرطوم.

النتائج: أظهرت الدراسة أن هنالك إنخفاض ذو دلالة معنوية في مستوى الكالسيوم(مستوى المعنوية 0.000) وأن هنالك إرتفاع ذو دلالة معنوية في مستوى الهرمون المضاد للميليريان (مستوى المعنوية 0.000) في عينات الإناث اللاتي يعانين من متلازمة تكيس المبايض مقارنة بعينات التحكم. كما أظهرت الدراسة أن هنالك إنخفاض غير معنوي في مستوى الاستراديول والالبيومين في عينات المرضى مقارنة بعينات التحكم (مستوى المعنوية (0.086 و 0.283))على الترتيب.

مما سبق ذكره يتضح أن متلازمة تكيس المبايض تقلل من مستوى الكالسيوم والاستراديول كما تزيد مستوى الهرمون المضاد للميليريان

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List of abbreviations

Symbol	Abbreviation
ACTH	Adrenocorticotropic hormone
AE	Androgen Excess
AES	Androgen Excess Society
AMH	Anti-Müllerian hormone hormone
BCG	Bromo Cresol Green
CAH	Classic congenital adrenal hyperplasia
CYP	Cytochrome P
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonuclic Acid
ECF	Extra Cellular Fluid
HCG	Human Chorionic Gonadotropin
IH	Iatrogenic hyperandrogenism
LH	Luteinizing hormone
mRNA	Massenger Ribonucleic acid
NADPH	Nicotin amide adenine dinucleotide phosphate
NCAH	Non-classic congenital adrenal hyperplasia
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
PCOS	Polycystic ovary syndrome
PTH	Para Thyroid Hormone
RANK	Receptor Activator of Nuclear factor B Ligand
RNA	Ribonucleic acid
SHBG	Sex hormone binding globulin
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for Social Sciences
TBG	Thyroxine-binding globulin

UTR	Untranslated region
VNTR	Variable Number of Tandem Repeats
Δ4A	Δ4-Androstendione

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1- Introduction, Rationale and Objectives

1.1 Introduction:

Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy of women, is a combination of chronic an ovulation or oligomenorrhoea and clinical or biochemical hyperandrogenism and ovarian polycystic changes observed by ultrasound (Belinda, 2016).affect about 6–8% of the population (Simon et al, 2013). In recent years, acceptance of the concept that PCOS is a heterogeneous disorder (that is, capable of having somewhat different manifestations in different people) and the exact cause not known until now, and it is a very common problem among patients attending infertility clinics. The diagnosis depend on above criteria and rule out other causes of hyperandrogenism. Recent study conducted to assess Serum Anti-Müllerian hormone as laboratory predictor in infertile women with PCOS. 17B-oestradiol is the principle hormone produce by the ovaries also synthesized in the placenta from androgens secreted by the fetal adrenal glands (William et al., 2012).

Vitamin D has an important roles in various parts of the body, especially in the bones. The active form of vitamin D plays an important role in bone metabolism, regulation of calcium equilibrium and cell differentiation and proliferation (William et al., 2012). Studies comparing vitamin D levels between patients with PCOS and healthy women with normal ovulation have yielded conflicting results.

1.2Rationale:

PCOS is a syndrome associated with increased ovarian and adrenal androgen secretions and hyperandrogenism and resultant hirsutism, hyperinsulinemia, Although vitamin D has important functions in the reproductive system. Vitamin D receptors are found in ovarian and endometrial tissues and play an important role in steroidogenesis. Vitamin D deficiency is more frequently encountered in patients with PCOS. On the other hand vitamin D primarily plays a role in bone metabolism and regulate calcium hemostasis, any depletion or disorder in vitamin D will result on serum calcium.

1.3 Objectives:

General objective:

To evaluate the possible changes in serum calcium, albumin and estradiol levels among females patients with polycystic ovarian syndrome.

Specific objectives:

1. To estimate the plasma level of calcium, albumin and estradiol among female patients with polycystic ovarian syndrome compared to apparently healthy female control group.
2. To correlate between plasma level of calcium and estradiol in female patients with polycystic ovarian syndrome.

2. Literature Review

2.1 Polycystic ovarian syndrome:

An international consensus definition of PCOS has defined patients with PCOS at least 2 of the following criteria: Oligomenorrhoea or amenorrhea. Clinical and/or biochemical signs of excessive androgen secretion. Presence of at least 12 follicles measuring 2-9mm in diameter, an ovarian volume >10ml, or both. Only one ovary needs to meet this criterion. Although ultrasound scan is therefore not essential to make the diagnosis. PCOs is very common having prevalence in women of child bearing age 5-10% and may be higher in women of South Asian origin. There is no single diagnostic criterion to confirm the clinical diagnosis. clinical manifestation include infrequent or absent menses, an ovulatory infertility, signs of androgen excess (hirsutism ,acne or amenorrhea) although the classical profile of PCOS is that of hyper secretion of LH and androgens with normal concentrations of FSH, a wide spectrum of findings are seen and abnormalities in LH are not always present. In addition to establishing the diagnosis, it is also important to exclude disorders with similar presenting features such as Classic congenital adrenal hyperplasia, Cushing's syndrome and androgen-secreting tumors. many women with PCOS have an increased risk of insulin resistance which, with the prevalence of obesity, is a powerful risk factor for progression to type 2 diabetes .They also have an increased long-term risk of endometrial hyperplasia /cancer (Simon *et al*., 2013).

Polycystic ovary syndrome (PCOS) is a common hormone disorder that affects approximately 5 million women of reproductive age in the United States. Women with PCOS have difficulty becoming pregnant (i.e., are infertile) and may have high levels of androgen hormones from the ovary and adrenal gland. In addition to fertility impairment, a woman with PCOS may have some of the following symptoms and findings:- Irregular or no menstrual periods in women of reproductive age (ovulatory dysfunction), acne, weight gain, excess hair growth on the face and

body, thinning scalp hair, ovarian cysts (polycystic ovarian Morphology) and Mental health problems. Women with PCOS are often resistant to the biological effects of insulin and, as a consequence, may have high insulin levels. Women with PCOS are at risk for type 2 diabetes, high cholesterol, and high blood pressure. Obesity also appears to worsen the condition. The degree of obesity may vary by ethnicity. In 1990, the National Institute of Health (NIH) held a conference on PCOS to create both a working definition of the disorder and diagnostic criteria. The outcome of this conference, the NIH Criteria, served as a standard for researchers and clinicians for more than a decade. In 2003, a consensus workshop in Rotterdam in the Netherlands developed new diagnostic criteria, the Rotterdam Criteria. The Androgen Excess (AE) and PCOS Society proposed the AE-PCOS Criteria in 2006 (David *et al.*, 2012).

Hyperandrogenism is very frequent in adolescent girls and is a source of concern for the girl herself, her family, and the clinician. Androgen excess during puberty produces a variety of clinical signs and symptoms that must be appropriately recognized, evaluated, and treated. Unfortunately for the pediatric endocrinologist, the criteria are so broad that many adolescents are presenting with transitory functional hyperandrogenism and menstrual disorders during puberty risk being misdiagnosed. The well known long-term sequelae of PCOS now present a challenge for pediatric endocrinologists to make an early diagnosis (in the pubertal period) and to treat these teenagers both symptomatically and prophylactically. The striking trend toward adolescent obesity should reinforce our responsibilities. We therefore propose to screen for PCOS all adolescents presenting oligo-amenorrhea within two years after menarche, particularly if hyperandrogenism is associated with low birth weight, family history of PCOS, abdominal obesity, and/or insulin resistance (Charles *et al.*, 2006).

This is a condition showing features of hyperandrogenism with anovulation and abnormal ovarian morphology and is the most common cause of anovulatory infertility. Presenting clinical symptoms may also include hirsutism, menstrual disturbances, enlarged polycystic ovaries and infertility. Plasma testosterone and androstenedione concentrations are often increased. The plasma LH may be elevated with normal FSH. Because plasma SHBG concentrations are reduced in obese individuals, the plasma concentration of free testosterone is often increased. The plasma prolactin concentrations may also be high. Multiple small sub capsular ovarian cysts may be demonstrated on ultrasound scanning of the ovaries. Polycystic ovary syndrome is also associated with insulin resistance, obesity and elevated plasma insulin concentrations, which may stimulate androgen production from the ovarian theca interna cells. Individuals may also have hyperlipidaemia, glucose intolerance and hypertension (Martin, 2012).

2.1.1-Androgens excess:

A patient with androgen excess has variable degrees of excess hair on the face, chest, abdomen and thighs, acne, and obesity. PCOS is clinically defined by hyperandrogenism with chronic an ovulation without underlying disease of the adrenal or pituitary glands. This syndrome characterized by infertility, hirsutism and obesity (in approximately half of those affected), and various menstrual disturbances from amenorrhea to irregular vaginal bleeding. Relatively low FSH concentrations and disproportionately high LH concentrations are common in PCOS. Serum androstenedione and testosterone (total and free concentrations) are elevated with mean concentration 50%to150% higher than normal. PCOS patient have substantial estrogen production because of the peripheral conversion of androgens to estrogen. The anovulation is caused by continuous estrogen stimulation of the endometrium (Carl *et al*., 2006).

2.1.1.1-Hirsutism:

Hirsutism is defined as the excessive growth of terminal hair in women and child in distribution similar to that occurring in post pubertal men (Carl *et al.*, 2006).

Clinical features of hyperandrogenism frequently seen in PCOS include hirsutism, acne, and androgenic alopecia. Here, we review the prevalence of these features in this disorder hirsutism is the presence of terminal hairs on the face and/or body in a female in a male-type pattern. The most common method of determining the presence of hirsutism uses a visual score. (Azziz *et al*., 2009). Various methods have been proposed. The most commonly used method is a modification of a method originally reported by Ferriman and Gallwey. Nine body areas, including the upper lip, chin, chest, upper back, lower back, upper and lower abdomen, upper arm, and thigh, are assigned a score of 0–4 based on the density of terminal hairs. A score of 0 represented the absence of terminal hairs, a score of 1 minimally evident terminal hair growth, and a score of 4 extensive terminal hair growths. The cutoff value should be established after the study of a large population of unselected women. Using this approach, cutoff values for defining hirsutism have been variously reported to be a score of 6 or greater, 7 or more, and 8 or more. (Azziz etal, 2009). However, we should note that the prevalence of hirsutism in PCOS will vary according to the race and ethnicity of the population being studied. These data suggest that the degree of body and terminal hair growth and the prevalence of hirsutism are not significantly different between unselected White and Black women. Consequently, it is likely that there will be little difference in the prevalence of hirsutism between Black and White PCOS women, although this remains to be confirmed. Consistent with the lower population prevalence of hirsutism observed in East Asian women, a comparative study of patients with PCOS from the United States (primarily Mexican Americans), Italy, and Japan noted that Japanese women had a significantly lower mean hirsutism score than their non-Asian counterparts.

However, the lesser prevalence of hirsutism among East Asian PCOS patients may not extend to all groups in the region. For example wijeyaratne and colleagues observed that hirsutism was more prevalent and more severe among PCOS patients of Southern Asian extraction (Pakistani, Bengali, Gujarati, or Dravidian Indian) than Whites. Likewise, among women of Indian descent in New Zealand, about two thirds of women with PCOS presented with clinical evidence of hirsutism, similar to the prevalence found in women of European, Maori, and Pacific Island descent. Although it is clear that there is racial variation in hair growth patterns, race-specific normative ranges have not been well established, which is required to determine whether a particular woman has excessive amounts of body of facial hair. Overall, hirsutism is an important feature of PCOS, affecting approximately 65% to 75% of patients with PCOS, including women of White, Black, and Southeast Asian race. The prevalence of hirsutism in PCOS is likely to be less among women of East Asian extraction (Azziz *et al*., 2009).

2.1.1.2-Acne:

Acne affects approximately 12% to 14% of white PCOS patients although the prevalence of this dermatologic abnormality varies with ethnicity: it is reportedly higher in Asian Indians and lower in Pacific Islanders. In a study of 248 women with PCOS in Italy, acne alone in the absence of other pilosebaceous features was present in 23.4%. Among 716 patients with PCOS, 14.5% presented with acne, either alone or in combination with hirsutism. In a prospective study of women presenting for blood donation, Asuncion and colleagues noted that of the 10 women diagnosed with PCOS, four (40%) had acne, three without associated hirsutism. However, various surveys have noted a relatively high prevalence of acne in the general population, particularly among younger women. Approximately 20% of individuals in their midteens and 15% of those in their early 20s complain of acne; even 10% of women in their 30s and 5% of women 40 to 60 years old will complaint of, albeit mild, acne.

Consequently, the degree to which PCOS increases the risk of acne above the general population prevalence is unclear. The variability in the prevalence of acne is compounded by the fact that there is no single scoring system used. Overall, although acne affects 15% to 25% of PCOS patients, it is unclear whether the prevalence of acne is significantly increased in these patients over that observed in the general population (Azziz *et al.,* 2009).

2.1.1.3-Androgenic alopecia:

Scalp hair loss in women is a distressing complaint with significant psychologic morbidity. It usually represents the pilosebaceous unit response to endogenous androgens and may be associated with acne and hirsutism. Androgen sensitivity of the pilosebaceous unit varies, and there is poor correlation between clinical features and evidence of biochemical hyperandrogenism. The presence of DHT, formed from the 5a-reduction of tin the dermal papilla, is associated with a higher 5a-reductase activity in the hairs plucked from a scalp presenting with androgenic alopecia. In addition to androgen excess, other potential etiologies of alopecia or diffuse scalp hair loss in any woman may be genetic (i.e., familial premature scalp follicular loss), environmental (e.g., damage following the use or abuse of hair cosmetics), and nutritional (e.g., poor protein intake, zinc deficiency, iron-deficient anemia). Androgenic alopecia is a recognized sign of PCOS. However, the prevalence of this abnormality in PCOS is unclear. Although we previously noted that PCOS patients may account for _10% to 40% of all women with alopecia, literature defining the incidence of alopecia in either normal women or women with PCOS is sparse. The pattern of hair loss in PCOS generally involves thinning of the crown with preservation of the anterior hairline. Androgenic related alopecia in women with PCOS tends to be seen in the anterior midvertex area extending to the crown. The anterior hairline remains intact in women with PCOS and significant a bit emporal scalp hair recession is unusual except in virilizing syndromes. Unfortunately, a loss

of at least 25% of scalp hair is needed before a woman becomes aware of thinning of her scalp hair. The sole presence of alopecia or diffuse scalp hair loss in women may be the sole dermatologic sign of PCOS (Azziz *et al.,* 2009).

2.1.1.4-Amenorrhoea:

Amenorrhoea can be primary (menstruation has never occurred) or secondary. Oligomenorrhoea is sparse or infrequent menstruation; it can be due to less severe forms of some of the causes of amenorrhoea. Primary amenorrhoea can occur as part of the syndrome of female hypogonadism, but can also be present in normally feminized women. The commonest cause of amenorrhoea in women of child-bearing age is pregnancy, and this possibility, however unlikely, must always be excluded. The finding of an apparently high plasma LH concentration may suggest pregnancy before a pregnancy test is performed: chorionic gonadotrophins cross-reacts in some assays for LH. Pregnancy apart, amenorrhoea in normally feminized women is most frequently due to a hormonal disturbance that results in a failure of ovulation. Causes include: disordered hypothalamo-pituitary function, related to weight loss (30–35% of cases in most series) or hyperprolactinemia (10–12%), but idiopathic in some 10% of cases, ovarian dysfunction (e.g. autoimmune disease leading to premature menopause) (10–12%), increased androgen production (particularly polycystic ovary syndrome (PCOS) and late-onset congenital adrenal hyperplasia) (30–35%). Weight loss can lead to a decrease in the frequency of the pulsatility of GnRH secretion and thus decreased secretion of LH and FSH. Menstruation almost always ceases if weight falls below 75% of the ideal, and may do so with smaller losses. Regular menstruation returns if weight is regained. Severe stress and intensive exercise regimens, such as are adopted by elite long-distance runners, ballet dancers and gymnasts, can also lead to amenorrhoea, probably for complex neuroendocrinological reasons in addition to any effect of decreased body weight. Amenorrhoea due to excessive androgen secretion is often associated with hirsutism

or even virilism. Uterine dysfunction is an uncommon cause of amenorrhoea. It can be excluded, if necessary, by the progestogen challenge test. If medroxyprogesterone acetate is given orally (10 mg daily for 5 days), the occurrence of vaginal bleeding 5–7 days later signifies that the uterus was adequately oestrogenized. If bleeding does not occur, the test is repeated, giving oestrogen (ethinyloestradiol, 50 mg daily for 21 days, with progestogen on the last 5 days). Absence of bleeding indicates uterine disease. If bleeding occurs, oestrogen deficiency is present. The diagnosis of hormonal causes of amenorrhoea requires basal measurements of plasma FSH, LH and prolactin concentrations. A high FSH concentration is indicative of ovarian failure (and is more sensitive in this respect than LH). If LH, but not FSH, is elevated, and the patient is not pregnant, the most likely diagnosis is PCOS, and pelvic ultrasonography should be performed. If LH and FSH concentrations are normal or low, a pituitary or hypothalamic disorder should be sought, by anatomical studies and dynamic testing of the hypothalamo-pituitary axis in a manner similar to that described for male hypogonadism. As in males, however, the results of such tests do not always distinguish between pituitary and hypothalamic disorders. The management of amenorrhoea depends on the cause, and whether fertility is required. In hyperprolactinemia, the treatment is directed to the underlying cause wherever possible (e.g. withdrawal of drugs, treatment of hypothyroidism). In ovarian, pituitary or hypothalamic disease, when fertility is not required, cyclical oestrogen and (if the patient has a uterus) progestogen replacement is given. In established ovarian failure, pregnancy is only possible using donated ova. If fertility is required in pituitary failure, treatment is with human FSH and LH; HCG may be required to mimic the mid-cycle LH peak and stimulate ovulation. Careful monitoring of plasma oestradiol concentrations is necessary to detect hyper stimulation, which carries a risk of multiple pregnancies and the production of ovarian cysts. Patients with hypothalamic disease may respond to clomiphene. This substance blocks oestradiol

receptors in the hypothalamus and may stimulate GnRH (and thus LH and FSH) secretion (Azziz *et al*., 2009).

Nonresponders are treated with pulsatile GnRH. Clomiphene is also useful in inducing ovulation in patients with PCOS. When it has not been possible to distinguish between hypothalamic and pituitary disease, a failure to respond to pulsatile GnRH sug gests that amenorrhoea is due to pituitary dysfunction (William *et al*., 2012).

Amenorrhea due to androgen excess can be due to adult onset CAH, corticotropin-dependent Cushing syndrome, or polycystic ovary syndrome (PCOS). Some individuals with 21-hydroxylase deficiency do not manifest any developmental abnormalities or salt wasting, but they present with signs of androgen excess. This clinical syndrome, referred to as nonclassic, adult-onset, or late-onset CAH, may be clinically indistinguishable from PCOS. Serum androstenedione and testosterone concentrations (total and free concentrations) are elevated, with mean concentrations 50 to 150% higher than normal. Abnormal bleeding patterns seen in PCOS are due to chronic anovulation and lack of progesterone stimulation and withdrawal. Chronic estrogen exposure without progesterone may predispose patients to endometrial cancer. Some attempt has been made to link PCOS to leptin, a hormone that is secreted by adipocytes and is thought to play a role in regulating food intake and metabolism. Animals that lack leptin are infertile; leptin injection increases gonadotropin secretion and restores fertility. For women with PCOS who wish to conceive, treatment is aimed at ovulation induction. Weight reduction should be attempted first in those women who are overweight, as it often helps to promote ovulation. If ovulation does not occur, then medications such as clomiphene citrate, metformin, and aromatase inhibitors may be useful. Ovarian hyperthecosis, a non-neoplastic lesion of the ovary characterized by the presence of islands of luteinized

thecal cells in the ovarian stroma, is sometimes confused with PCOS (Carl et al, 2006).

2.1.1.5Infertility:

Infertility is a common clinical problem, leading approximately one in six couples in the UK to seek professional advice. Investigation is usually considered appropriate when a couple has been unable to conceive after 12 months of trying, assuming regular, unprotected intercourse. It can be primary (conception has never occurred) or secondary, and due to problems affecting either the male or the female. Ovulatory failure, due most frequently to hyperprolactinemia or hypothalamic–pituitary dysfunction, is responsible in approximately 20% of cases, and defective sperm production in about one-quarter. Endocrine causes of infertility are rare in males. A couple in their late 20s was infertile in spite of regular intercourse over a two-year period. Each partner had a child by a previous marriage. The woman's periods had recently become irregular. A semen sample contained a normal count of motile sperm. Physical examination revealed no abnormality. The woman was on steroid replacement treatment for adrenal failure, which had been diagnosed in her late teens (William *et al.*, 2012).

Infertility can result from ovulatory or uterine problems; mechanical problems, including obstruction of the fallopian tubes; male fertility factors; or multiple factors in either sex or combined female and male factors. Ovulatory problems are the most common cause of female infertility. Polycystic ovarian syndrome (PCOS) affects up to 5% of reproductive-age women. It is the most common cause of ovulatory infertility. PCOS is a condition characterized by multiple ovarian cysts, often found in a row, resembling a "string of pearls." Ovarian cysts are fluid-filled sacs arising from follicles swollen with fluid that are prevented from producing mature oocytes. Patients with PCOS also have hormonal

imbalances, including decreased levels of LH, FSH, and progesterone and increased androgen production, including excess testosterone and DHEAS causing hirsutism or male facial patterns of hair growth. Insulin resistance is a common associated condition. PCOS is generally diagnosed when two of the following three criteria are present and other possible causes can be ruled out: clinical or laboratory results showing excess androgen secretion, decreased or absence of ovulation, and ovaries found by imaging techniques such as ultrasound to contain many cysts. Although the exact etiology of the problem is still unknown, genetic factors may be involved. Around the time of menopause, impairment of ovulation may cause infertility with adverse effect on follicle size and oocytes quality despite regular ovulation and normal gonadotropin levels. These factors are considered when treating older women with infertility. Serum levels of LH, FSH, and inhibin A and B may be helpful in assessing infertility and treatment options. Infertility diagnostic testing is as varied as treatment options. The patient workup for infertility includes a careful, detailed history, which can help to limit the number of laboratory tests required. Availability of tests varies from center to center, so availability is one of the considerations for infertility testing. Typical laboratory tests ordered are FSH on day three of the ovulatory cycle, LH, estradiol, prolactin, and TSH levels. Measurement of ovarian and adrenal androgens such as testosterone and DHEAS should be decided on the basis of ovulatory status of the patient and the clinical picture. (Wendy, 2007).

Infertility is defined as the inability to conceive after 1 year of unprotected intercourse. It has been estimated that 93% of healthy couples practicing unprotected intercourse should expect to conceive within 1 year, and 100% will be successful within 2 years. a specific cause of infertility is identified

in ≈80% of couples: one third are due to female factors alone, one third to male factors alone, and one third to a combination of problems. Primary infertility refers to couples or patients who have had no previous successful pregnancies. Secondary infertility encompasses patients who have previously conceived, but are currently unable to conceive. These types of infertility generally share common causes. Infertility problems often arise as a result of hormonal dysfunction of the hypothalamic-pituitary-gonadal axis. Measurements of peptide and steroid hormones in the serum are therefore essential aspects of the evaluation of infertility. This section focuses on hormonal and biochemical aspects of evaluating infertility (Carl *et al*., 2006).

2.1.2-Genetic of Polycystic Ovarian Syndrome:

The mode of inheritance of PCOS remains unknown, and recent studies indicate that this disorder could be a complex trait. This means that several genes are interacting with environmental factors to provoke the phenotype. In contrast, biochemical parameters, including fasting insulin levels or hyperandrogenemia, seem to be highly heritable parameters, suggesting that some clinical signs, symptoms, or biochemical parameters of PCOS could be transmitted as Mendelian autosomal dominant or X-linked traits, but the genetic studies have not as yet concluded the pattern of heredity. While studies, so far, are unable to exclude an autosomal or X-linked dominant mode of inheritance, the heritability of PCOS is probably more complex, similar to that of type 2 diabetes mellitus or cardiovascular disease (Wendy, 2007). However, a positive family history appears to be the most informative risk factor for the development of PCOS. Furthermore, environmental factors alter the clinical and

biochemical presentation in those with genetic predisposition to PCOS. A relation between PCOS with the X chromosome aneuploidies and polyploidies in addition to other cytogenetic abnormalities has been confirmed. Some of the cases of PCOS may represent an intermediate condition in a spectrum that extends from the streak gonad of Turners syndrome to the normal ovary. The concept is that at least some cases of PCOS may be due to X chromosomal factors causing an abnormal follicular apparatus. In addition, large deletion of the long arm of chromosome 11 was seen in some of the PCOS cases. However, there is no large cytogenetic study to identify karyotype abnormalities. There are different candidate genes as a cause of PCOS; such as genes involved in steroid hormone synthesis and action, genes involved in carbohydrate metabolism and fuel homeostasis, genes involved in gonadotropin action and regulation; and genes in the major histocompatability region, which could account for certain PCOS features (Wendy, 2007).

Increased androgen secretion and insulin resistance persist in cultured theca cells and skin fibroblasts, respectively, from women with PCOS, which suggest that these are intrinsic, presumably genetic, defects. Different studies have indicated a genetic susceptibility to PCOS. It was shown that polycystic ovaries and hyperandrogenemia are present in 50% of sisters of affected women. Therefore genetic analyze of candidate genes have been performed. Both linkage and association studies have suggested that PCOS can be explained by the interaction of a small number of key genes with environmental, particularly nutritional factors. Hyperandrogenemia is genetically determined and the result of familial studies indicating that hyperandrogenism clusters as a dominant genetic trait. The steroid synthesis gene CYP11a, coding for P450 cholesterol side chain cleavage and the insulin gene regulatory region may be involved. However, it is unlikely that the hyperandrogenemia of PCOS is principally determined by polymorphisms or mutations in the genes encoding a single steroidogenic enzyme activity, such as

CYP17 or CYP11a. In addition, an increase of mRNA abundance in PCOS has been found in corresponding to the genes of aldehyde dehydrogenase-6 and retinol dehydrogenase-2, which both increases the expression of 17a-hydroxylase. Recent studies have found a significant prevalence of CYP21 mutation, gene encode the 21-hydroxylase enzyme mimic the PCOS phenotype, in the supposed PCOS population (Sheikhha *et al*., 2007).

The first step in steroidogenesis is the conversion of cholesterol into progesterone, catalyzed by the P450 Cytochrome side chain cleavage enzyme encoded by CYP11a gene located at 15q. Investigation of CYP11A gene showed a significant association between serum testosterone levels and the alleles of the CYP11a with a 5' untranslated region (UTR) consisting of repeats of a (tttta) n pent nucleotide, a variable number tandem repeat (VNTR) polymorphis. Further investigation is required due to these controversial results in order to confirm a role in the a etiology of PCOS of this gene. Another part in steroidogenesis is the conversion of 17-hydroxyprogesterone into 11-deoxycortisol which is catalyzed by the 21-hydroxylase enzyme encoded by CYP21. The deficiency of this enzyme is responsible for most cases of congenital adrenal hyperplasia and increased serum 17-hydroxyprogesterone levels are correlated with its deficiency. It is a common finding among women with functional hyperandrogenism or PCOS an increased serum 17-hydroxyprogesterone response to ACTH stimulation. Furthermore, patients having both heterozygote CYP21 mutations and clinical symptoms exhibit a PCOS-like phenotype. Accordingly, mutations of CYP21 have been investigated as a candidate gene in patients with PCOS. Two studies showed that children with premature pubarche and adolescent girls with hyperandrogenism were heterozygous for mutations in CYP21. On the other hand, there are other researchers that found no clear concordance between the CYP21 genotype and the functional origin of androgen excess. Overall, CYP21 and associated mutations do not seem to play a

key role in the development of PCOS. The conversion of pregnenolone and progesterone into 17-hydroxypregnenolone and 17-hydroxyprogesterone, respectively and of these steroids into dehydroepiandrosterone (DHEA) and Δ4-Androstendione (Δ4A) is catalyzed by the P450c17α enzyme. This enzyme has both 17α-hydroxylase and 17, 20-lyase activities and is encoded by CYP17 located at 10q. It was reported increased P450c17α expression and enzymatic activity in ovarian theca cells from women with PCOS as well as increased transactivation of the CYP17 promoter. Moreover, it was showed that CYP17 expression is dysregulated at the level of mRNA stability in PCOS theca cells. Another study identified a rare T/C single nucleotide polymorphism (SNP) in the promoter region of CYP17 increasing the susceptibility to develop PCOS (Sheikhha *et al*., 2007). Subsequently, more comprehensive studies have failed to detect a significant linkage between CYP17 and PCOS. Although CYP17 gene does not seem to be a candidate gene in the pathophysiology of PCOS, it should be noted that post-translational regulation of this gene product might play a role in the pathophysiology of PCOS. The enzyme complex aromatase converts androgens to estrogens. This enzyme complex is composed of the Cytochrome P450 aromatase and the NADPH Cytochrome P450 reductase, and P450arom is encoded by CYP19 located at 15p. Aromatase deficiency has been reported in a number of hyper androgenic patients. It has been demonstrated that granulosa cells obtained from medium-sized follicles of women with PCOS have little aromatase activity. Similarly, it has been showed that when compared to the control follicles, all PCOS follicle contained low levels of P450arom mRNA, estradiol, and lower aromatase stimulating bioactivity. These findings indicate that the aromatase activity might be decreased in PCOS follicles, and that the possible androgen excess resulting might contribute to abnormal follicle development. Association studies utilizing SNPs and haplotypes showed association with PCOS symptoms and serum testosterone levels (Prapas *et al.*, 2009).

2.1.3-Diagnositic Criteria of Polycystic Ovarian Syndrome:

In 1990, the first formal attempt to consolidate a clinical definition of PCOS by the National Institute of Child Health and Human Development resulted in PCOS being defined as the combined presence of Clinical and/or biochemical signs of hyperandrogenism and Oligo- or chronic anovulation in the absence of all other reasons for anovulatory infertility. The NICHD criteria were deliberately listed in order of perceived importance. The use of these criteria defined PCOS as a syndrome whose primary determinant was a derangement in androgen homeostasis with consequent effects on menstrual cyclicity. Ultrasonographic evidence of polycystic ovaries was concluded to be "suggestive" of PCOS but not necessarily diagnostic. This prevailing opinion reflected the paucity of British and European attendees at the meeting to define the NICHD criteria, because Ultrasonographic evidence of polycystic ovaries had long been considered definitive evidence of PCOS in the UK and most of Europe. The NICHD criteria represented a very important first step towards establishing a universally accepted clinical definition for PCOS. However, it is important to recognize that the criteria were based on majority opinion and not clinical trial evidence. In the years that followed, it became apparent that the clinical presentation of PCOS was much more variable than that described by the NICHD criteria, and that polycystic morphology of the ovaries was a consistent finding in women demonstrating biochemical and clinical evidence of the syndrome (Marla *et al*., 2008).

In 2003, the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine amended the consensus criteria to include polycystic ovaries as a third diagnostic

marker and to allow for a diagnosis of PCOS if two of three criteria were met Oligo- or chronic anovulation, Clinical and/or biochemical signs of hyperandrogenism and Polycystic ovaries, in exclusion of other etiologies of androgen excess and anovulatory infertility is necessary. These "Rotterdam criteria" were intended to broaden the phenotypic expression of the syndrome and to redefine PCOS as primarily a syndrome of ovarian dysfunction. The Rotterdam criteria are controversial. Fulfilling two of three diagnostic criteria implies that PCOS can be diagnosed in the absence of androgen excess or menstrual irregularity the very factors that were once considered absolute requisites for the syndrome. While most agree that PCOS exists as a spectrum, it has been difficult to reconcile the absence of androgen excess in the diagnosis (Marla *et al*., 2008).

In 2006, the Androgen Excess Society formed a task force to review existing data on the phenotypic expression of PCOS and Patient demonstrates both: Hirsutism and/or hyperandrogenemia and Oligo-anovulation and/or polycystic ovaries and exclusion of other etiologies of androgen excess and anovulatory infertility are necessary. The AES concluded that although there was good evidence for features of PCOS (e.g., mild insulin resistance and mild ovarian dysfunction) in women with polycystic ovaries, androgen excess, and regular menstrual cycles, there was conflicting evidence supporting the presence of such features of PCOS in women with polycystic ovaries and ovulatory dysfunction but without clinical or biochemical signs of hyperandrogenism. The AES has proposed a new set of diagnostic criteria that acknowledge the wide prevalence of morphologic polycystic ovaries and the wide heterogeneity of PCOS. They do not, however, recognize a mild variant of the syndrome

in which little is known about metabolic status or long-term health risks (Marla *et al*., 2008).

All current definitions of PCOS require that other disorders of androgen excess or ovulatory function be excluded. Principally, the former includes 21-hydroxylase-deficient non-classic congenital adrenal hyperplasia (NCAH), Cushing's syndrome, androgen-secreting neoplasms (ASNs), and drug-induced or iatrogenic hyperandrogenism (IH): the latter includes thyroid dysfunction and hyperprolactinemia. NCAH is excluded by the measurement of a follicular phase (pre-ovulatory) basal 17ahydroxyprogesterone (17-HP) level, which if >2–4ng/ml mandates an acute adrenocorticotropic hormone (ACTH) stimulation test. Alternatively, evaluation for Cushing's syndrome, ASNs, or IH should be instituted if the history and physical exam suggests their possibility .Thyroid dysfunction and hyperprolactinemia can be excluded by the routine measurement of thyroid-stimulating hormone (TSH) and prolactin levels, although the prevalence of these disorders in women with overt hyperandrogenism is relatively low. Overall, patients being evaluated for PCOS should, at a minimum, have NCAH excluded by a basal 17-HP level, and possibly thyroid dysfunction and hyperprolactinemia, by TSH and prolactin levels (Bradley *et al*., 2007).

2.2-Estradiol:

The principal ovarian hormone is 17β-oestradiol.Oestrogens are also secreted by the corpus luteum and the placenta. Oestrogens are responsible for the development of many female secondary sexual characteristics. They also stimulate the growth of ovarian follicles and the proliferation of uterine endometrium during the first part of the menstrual cycle. They have important effects on cervical mucus and vaginal epithelium, and on other functions associated with reproduction. Plasma

concentrations of oestrogens are low before puberty. During puberty, oestrogen synthesis increases and cyclical changes in concentration occur thereafter until the menopause, unless pregnancy occurs. After the menopause, the sole source of oestrogens is from the metabolism of adrenal androgens; plasma concentrations fall to very low values. In the plasma, oestrogens are transported bound to protein, 60% to albumin and the remainder to SHBG. Only 2–3% remains unbound. Oestrogens stimulate the synthesis of SHBG and also that of other transport proteins, notably thyroxine-binding globulin (TBG) and transcortin, and thus increase total thyroxine and total cortisol concentrations in the plasma. Slowly rising or sustained high concentrations of oestrogens, together with progesterone, inhibit pituitary gonadotrophin secretion by negative feedback, but the rapid rise in oestrogen concentration that occurs prior to ovulation stimulates LH secretion (positive feedback). Oestradiol is present in low concentrations in the plasma of normal men. Approximately one-third is secreted by the testes, the remainder being derived from the metabolism of testosterone in the liver and in adipose tissue (Bradley *et al*., 2007).

The stimulated corpus luteum secretes large amounts of oestrogens and progesterone, but after six weeks the placenta becomes the major source of these hormones. There is a massive increase in the production of oestriol during pregnancy, but production of oestrone and oestradiol increases also. Oestradiol is synthesized in the placenta from androgens secreted by the fetal adrenal glands. Its measurement in maternal plasma or urine was formerly used to assess feto-placental function, but now has been superseded by ultrasonography, which can be used to provide direct measurements of fetal growth and placental blood flow. The same applies to measurements of other placental products, for example human placental lactogen and placental alkaline phosphatase (a heat-stable isoenzyme), which have been used in the past as indicators of placental function (William *et al.*, 2012).

Estrogens are secreted by the ovarian follicles and by the placenta in pregnancy (and to a much lesser extent by the adrenal glands and testes). Estrogen promotes development and maintains the female reproductive system, including the uterus, fallopian tubes, and vagina. It is responsible for development and maintenance of secondary female sex characteristics. Estrogen peaks at midcycle, causing a decrease in FSH but promoting the LH surge at midcycle. There are three primary estrogens: estradiol-17, estrone, and estradiol. Estradiol is the principal estrogen synthesized by the ovaries. Hyperprogesteronemia Prevents menstrual cycle from occurring and Causes infertility, abortion of fetus (Anna *et al*., 2010).

Dynamic changes in circulating estradiol level, including increase at menarche and decrease at menopause, occur in a woman's lifetime. Circulating estradiol levels decrease drastically during the menopausal transition, though the levels differ among races. It has been reported that estradiol levels in both Japanese and Chinese women were lower than those in Caucasians, Hispanic and African- Americans. This dynamic decrease in estradiol level induces menopausal symptoms, such as hot flashes and night sweat, urogenital symptoms, osteoporosis, coronary heart disease, stroke and possibly early onset of Alzheimer's disease in postmenopausal women. However, not only estrogen but also other endocrinological hormones may be involved in the occurrence of these diseases. Little attention has been paid to roles of endogenous androgens in women despite the results of studies suggesting that androgens may play important roles. Androgens are known to be important for normal physiology in women and to play key roles in the physical, sexual and emotional well-being of women. Therefore, it is necessary to take account of androgens as well as estrogen when considering women's health (Yasui *et* al, 2012).

2.3 Calcium:

2.3.1Physiology:

Calcium is the fifth most common element and is the most prevalent cation in the

human body. A healthy adult contains approximately 1–1.3 kg of calcium, and 99% of this is in the form of hydroxyapatite in the skeleton. The remaining 1% is contained in the extracellular fluid (ECF) and soft tissues. Additionally, less than 1% of the skeletal content of calcium is in bone fluid and exchanges freely with the ECF (Mundy, 1999).

2.3.2Biochemistry:

Serum (plasma) calcium exists in three distinct forms: (1) free or ionized calcium, which is the physiologically active form, accounting for approximately 50% of the total serum calcium; (2) complexed calcium, which is bound tightly to a variety of anions, including bicarbonate, lactate, phosphate, and citrate, accounting for approximately 10%; and (3) plasma protein-bound calcium, accounting for approximately 40%. Both ionized calcium and the calcium complexes are freely dialyzable. Approximately 80% of the protein-bound calcium fraction is associated with albumin. Because ionized calcium binds to negatively charged sites on the protein molecules, there is competition with hydrogen ions for binding sites on albumin and other calcium-binding proteins, and its binding is pH dependent. Although total serum calcium levels may remain unchanged, the relative distribution of the three forms is altered as a result of pH changes in ECF. Alkalosis promotes increased protein binding, with a subsequent decrease in free calcium, whereas acidosis decreases protein binding, causing an increase in free calcium levels. Because calcium is bound to proteins, total calcium levels are also altered by plasma protein concentration (Mundy, 1999).

2.3.3Functions:

In addition to its obvious importance in skeletal mineralization, calcium plays a vital role in such basic physiologic processes as blood coagulation, neural transmission, plasma buffering capacity and enzyme activity, and in the maintenance of normal

muscle tone and excitability of skeletal and cardiac muscle. It is an activator of intracellular signal transduction processes and is essential for DNA and RNA biosynthesis. Calcium is also involved in glandular synthesis and in regulation of exocrine and endocrine glands, as well as in the preservation of cell membrane integrity and permeability, particularly in terms of sodium and potassium exchange Sokoll et al, 2004).

2.3.4Absorption:

Calcium is absorbed in the duodenum and upper jejunum via an active transport process. Less than half of dietary calcium is absorbed in adults. However, calcium absorption increases during periods of rapid growth in children, in pregnancy, and during lactation. It decreases with advancing age. The major stimulus to calcium absorption is vitamin D. Calcium absorption is also enhanced by growth hormone, an acid medium in the intestines, and by increased dietary protein. The ratio of calcium to phosphorus in the intestinal contents is also important, because a ratio greater than 2:1 results in the formation of insoluble calcium phosphates and tends to inhibit calcium absorption. Phytic acid, derived from various cereal grains, can also form insoluble calcium compounds, as can dietary oxalate and fatty acids. Cortisol and excessive alkalinity of the intestinal contents are both inhibitory to calcium absorption (Sokoll et al, 2004).

2.3.5Homeostasis:

Ionized calcium concentration of the ECF is kept constant within a narrow range of approximately 1.25μmol/L. It is the ionized calcium concentration of the ECF that is the primary determinant of the hormonal influences that exert effects on ECF calcium levels. These effects are sometimes achieved at the expense of bone integrity. Adjustment of the ionized calcium concentration of the ECF is achieved

mainly by the actions of PTH and active 1, 25-dihydroxyvitamin D3 (1, 25[OH] 2D3), and calcitonin plays a smaller yet significant role. The principal target organs of these hormones are bone, kidney, and intestine. When plasma-ionized calcium concentration decreases, the parathyroid glands sense the change via membrane-bound calcium sensor protein and secrete PTH immediately (Watts. 1999).

Although parathyroid hormone has no direct effect on osteoclasts, it stimulates osteoblasts and their precursors to produce RANKL (the receptor activator of nuclear factor κB ligand). This substance, a member of the tumor necrosis factor superfamily, activates its receptor, RANK, which is expressed on osteoclasts and their precursors. This, in turn, promotes osteoclast formation and activity, and prolongs osteoclast survival by suppressing apoptosis. This explains why bone formation and bone resorption are coupled in normal bone physiology. The resorption of bone matrix releases calcium and phosphate into the ECF. At the same time, PTH also acts on the kidney to stimulate increased urine phosphate excretion and some calcium reabsorption in the distal nephron, returning the ionized calcium concentration to normal. It has been suggested that sufficient action of 1, 25(OH) 2D3 is mandatory for these steps to work appropriately. The kidney is almost exclusively responsible for this vitamin D activation. Calcitonin may play a role in the regulating process, although its significance in humans is controversial. Other hormones that affect calcium metabolism but whose secretions are not primarily affected by changes in plasma calcium and phosphate include thyroid hormone, growth hormone, adrenal glucocorticoids, and gonadal steroids (Watts, 1999).

2.4Albumin:

2.4.1Physiology:

The single most abundant protein in normal plasma is albumin, usually constituting up to two thirds of total plasma protein. For this reason, depressions in albumin level due to impaired synthesis (e.g., malnutrition, malabsorption, hepatic dysfunction) or to losses (e.g., ascites, protein-losing nephropathy or enteropathy) result in serious imbalance of intravascular oncotic pressure. This loss is manifested clinically by the development of peripheral edema. However, the congenital absence of albumin (analbuminemia) generally does not lead to such problems, presumably because of lifelong compensatory mechanisms that control hydrostatic pressures (Peters, 1977).

2.4.2Functions:

Albumin serves as a mobile repository of amino acids for incorporation into other proteins. A second function ascribed to albumin is that of a general transport or carrier protein. Many organic and inorganic ligands (e.g., thyroxine, bilirubin, penicillin, cortisol, estrogen, free fatty acids, warfarin [Coumadin], calcium, magnesium, heme) are complexed with different regions of the albumin molecule in covalent (e.g., δ-bilirubin) or dissociable binding (Lauff, 1982). These binding interactions with very different ligands are possible because of a wide variety of binding sites on the albumin molecule, which consists of 585 amino acids arranged in nine loops held together by the disulfide bonds between cysteine residues.

The primary sequence of albumin contains three major regions with three peptide loops each, suggesting that it arose from gene duplication of some ancestral gene in a tandem rearrangement process (Peters, 1977).

2.4.3Clinical significance:

Clinical measurements of albumin are very frequent, with determinations of total protein and albumin often included in chemistry panel profiles. Organ- or disease-oriented panels of chemistry tests in current use include the following measurements: comprehensive metabolic panel has albumin and total protein; renal function panel has albumin; hepatic function panel has albumin and total protein.

Elevations of serum albumin concentration are infrequent, although they do occur in dehydration as the plasma water phase shrinks. Following rehydration, the albumin level should fall to within the normal reference range. Elevation of serum albumin may also occur artifactually as the result of prolonged application of a tourniquet for venipuncture. In this instance, increased hydrodynamic pressure from venous backup forces water and small solutes out of the intravascular space, thereby concentrating cellular elements, micellar forms of lipoproteins, and proteins such as albumin (Delanghe, 2001).

Depression of albumin concentrations is frequent in sick individuals, and a review of hospitalized patients reveals that a substantial proportion of albumin measurements are below healthy reference ranges. Although some of these decreases are likely dilutional, owing to the administration of intravenous fluids, others are caused by loss of albumin into urine, ascitic fluid, or the gastrointestinal tract in enteropathies, or by decreased synthesis in the liver caused by hepatic disease such as cirrhosis or by secondary effects on synthesis resulting from compromised nutrition or diversion of synthetic capacity to other proteins. This sensitive but nonspecific reduction of albumin in so many different conditions has led to its being termed a “negative acute phase reactant”. Measurements of albumin concentrations are vital to the understanding and interpretation of calcium and magnesium levels because these ions are bound to albumin, and so decreases in albumin are directly responsible for depression of their concentrations too. In some disease states, decreases in albumin are at least partially compensated for by increases in other

serum proteins, thereby stabilizing oncotic pressures intravascularly. In particular, cirrhosis shows a major polyclonal increase of immunoglobulin in the γ-fraction (Minchiotti, 2008).

3. Materials and Methods

3.1. Study design:

This is facility based cross sectional study.

3.2 study area and duration:

This study was conducted in Khartoum state at Al sir abo al Hassan medical center during 2017.

3.3 Study population:

Forty Sudanese female patients with polycystic ovarian syndrome were selected for this study and other 40 healthy individual as control group.

3.4 Inclusion criteria:

Patients with polycystic ovarian syndrome who voluntarily accepted to participate in this study were included in this study.

3.5 Exclusion criteria:

Patients who refused to participate in this study, in addition to those with any menopausal and women receiving contraceptives were excluded.

3.6 Ethical consideration:

All individual included in this study told for study purpose and asked for agreement and verbal Consent was taken regarding acceptance to participate in the study and re-assurance of confidentiality. Before the specimen was collected.

3.7 Data collection:

Data were collected using a structural interviewing questionnaire, which was designed to collect and maintain all valuable information concerning each case examined.

3.8 Sample collection and processing:

About 2.5 ml of venous blood were collected from each participant (both case and control). The sample collected under aseptic conditions and placed in sterile lithium heparin containers and centrifuged for 5 minutes at 3000 RPM to obtain plasma then they obtained sample were kept in plain containers at 2-8 C0 until the time of analysis.

3.9 Estimation of estradiol by using ELISA method:

3.9.1 Principle of method:

In which one of the reaction components is bound to a solid-phase surface. In this Technique, an aliquot of sample is allowed to interact with the solid-phase antibody. After washing, a second antibody labeled with enzyme is added to form an Ab–Ag–Ab–enzyme complex. Excess free enzyme–labeled antibody then is washed away, and the substrate is added; the conversion of substrate is proportional to the quantity of antigen (Carl *et al*., 2006).

3.9.2 Procedure of estradiol measurement (Appendix II).

3.10 Estimation of total calcium by Arsenazo III Colorimetric method:

3.10.1 Principle of method:

Arsenazo III reacts with calcium to form a calcium-indicator complex usually measured at near 650 nm. The stable reagent exhibits high specificity for calcium at slightly acidic pH, arsenazo III has a high affinity for calcium ions, but the amount of complex formed is strongly pH dependent. Usually, imidazole buffers at pH 6.0 are employed for this reaction. (Nauck 2002).

3.10.2 Procedure of calcium measurement (Appendix III).

3.11 Estimation of Albumin by bromocresol green Colorimetric method:

3.11.1 Principle of method:

Albumin binds with bromcresol green (BCG) to produce a blue-green color with an absorbance maximum at 628 nm. The intensity of the color produced is directly proportional to the albumin concentration in the sample (Bishop et al 2000).

3.11.2 Procedure of Albumin measurement (Appendix IV).

3.12 Statistical analysis:

Data was analyzed to obtain means standard deviation and correlation of the sampling using statistical package for social science (SPSS) computer Programmed version 15, t test and person correlation were used for comparison and correlation.

4- RESULT

4-1 Demographical data:

The number of participants in this study were 80 individuals (40 female patients with polycystic ovarian syndrome and 40 healthy group) with the mean age of patients was (23 years) and the mean age of healthy group was (24 years) (table 4-1).

4.2 The mean levels of serum calcium, albumin, AMH and estradiol among patients with polycystic ovarian syndrome compared to control group:

Our study showed that serum calcium was found highly significant decreased in polycystic ovarian syndrome patients when compared to control group (P.value 0.000), where the reverse was true for albumin and estradiol which show insignificant decrease (P.value0.283) and (P.value0.086) respectively, while it showed that serum AMH was found highly significant increased in patients as compared to normal group (P.value 0.000) (table 4-2).

4.3 Correlations:

Our study showed that there was no correlation between serum calcium and estradiol levels among polycystic ovarian syndrome patients, also there was no correlation between calcium and AMH levels among polycystic ovarian syndrome patients, and no correlation between estradiol and AMH levels. There was significant correlation between serum calcium and albumin levels among polycystic ovarian syndrome patients (Figure (4-1), (4-2), (4-3) and (4-4) respectively).

Table 4-2: The mean levels of serum calcium, albumin, AMH and estradiol among patients with polycystic ovarian syndrome compared to control group:

Variable	PCOS N=40 Mean±SD	Control N=40 Mean±SD	*P.Value*
AMH ng/ml	10.6±5.92	1.4±0.22	0.000
Calcium mg/dl	7.0±0.69	9.2±0.55	0.000
Albumin g/dl	4.1±0.56	4.5±0.46	0.124
Estradiol pg/ml	86±39	101±38.6	0.086

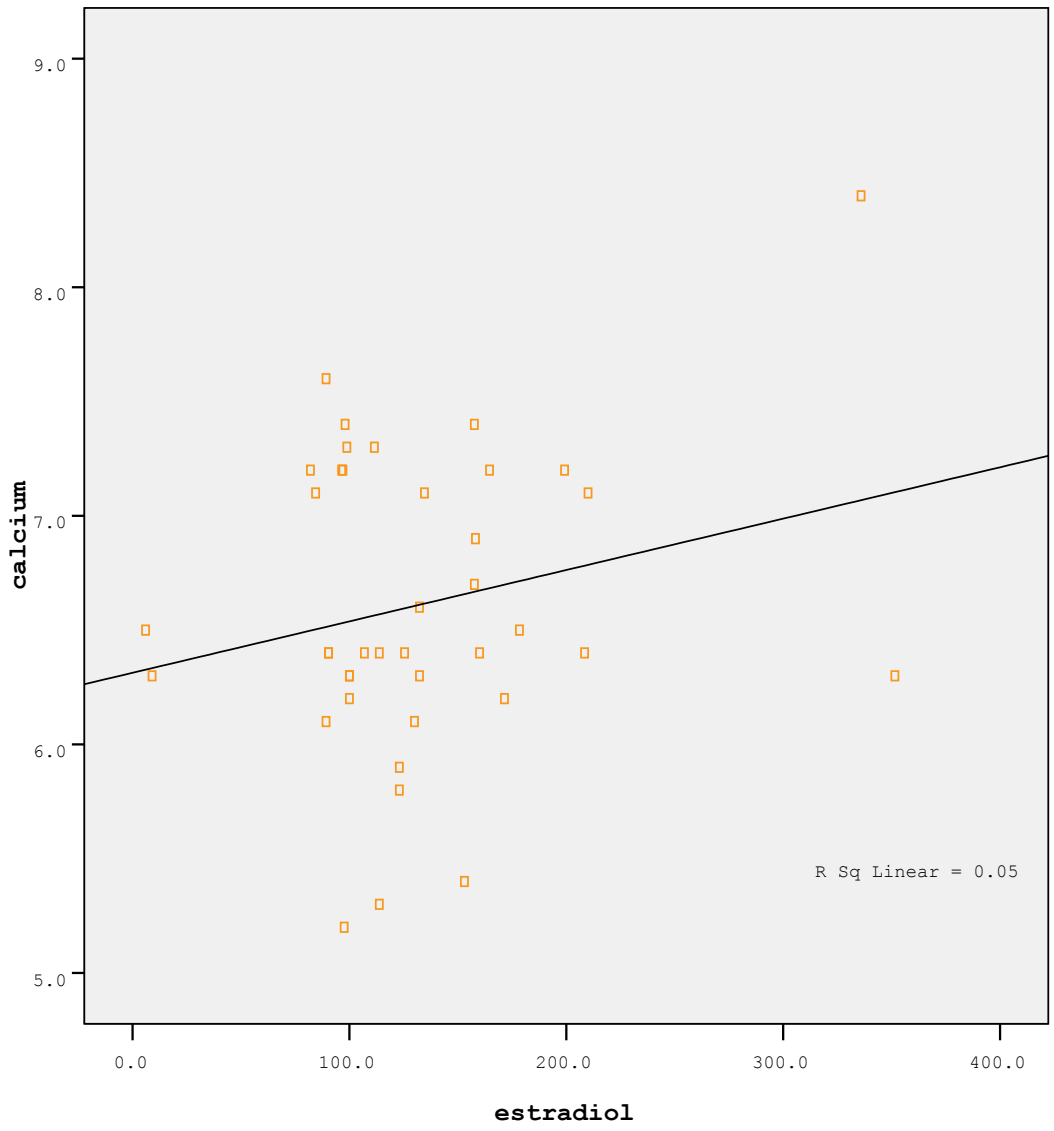

Figure (4-1): Show correlation between calcium and estradiol (r =0.125, p.value 0.441)

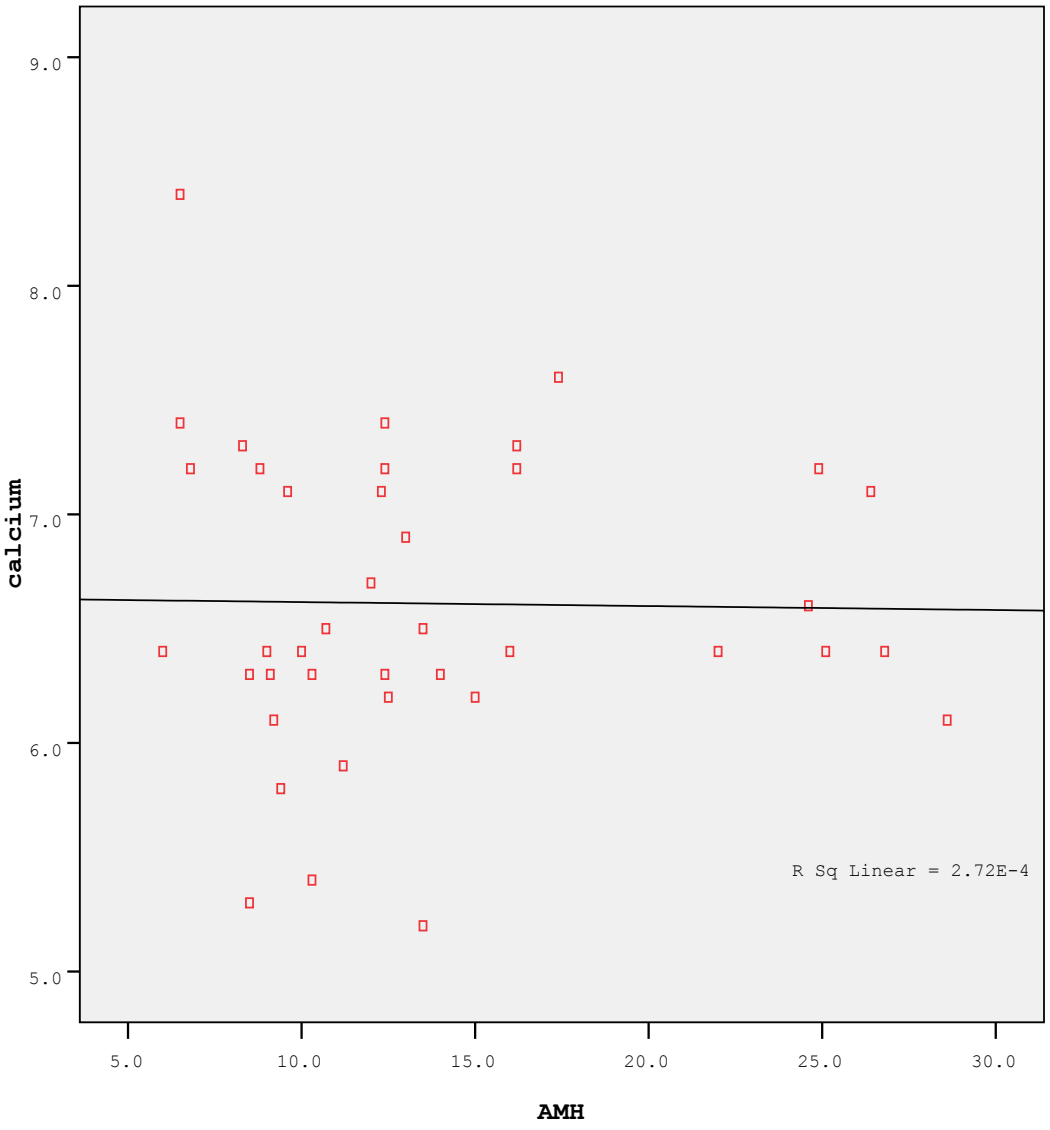

Figure (4-2): Show correlation between calcium and AMH (r =-0.421, p.value 0.321)

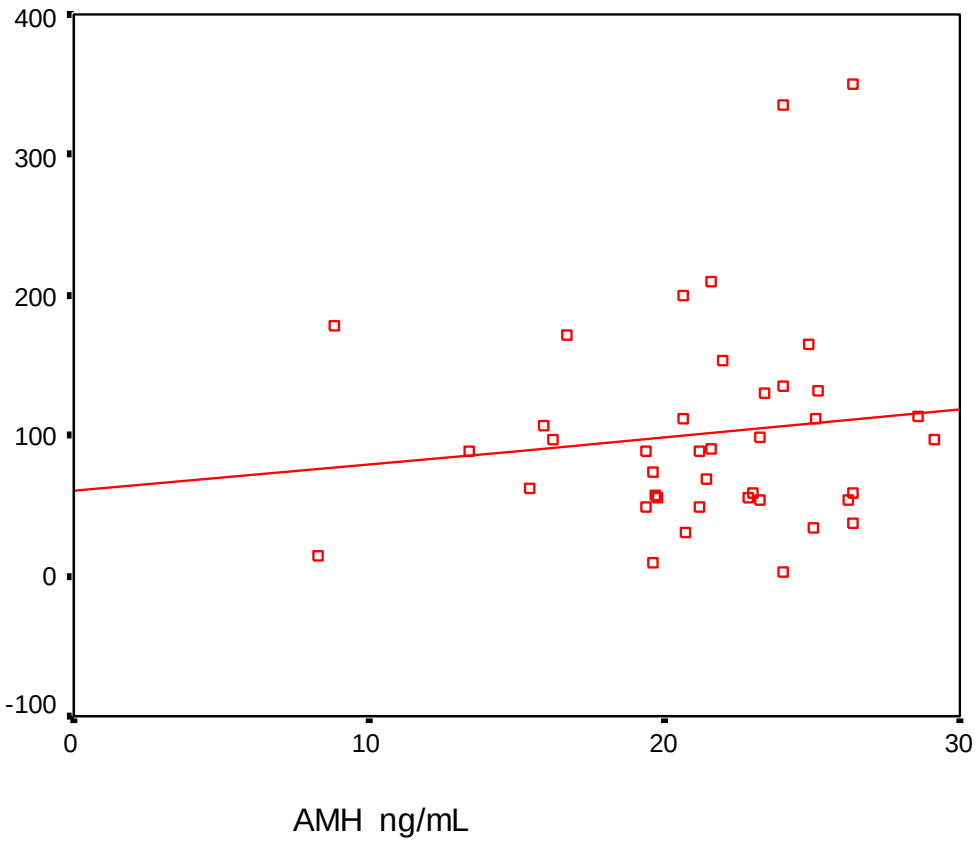

Figure (4-3): Show correlation between estradiol and AMH (r =-0.010, p.value 0.927)

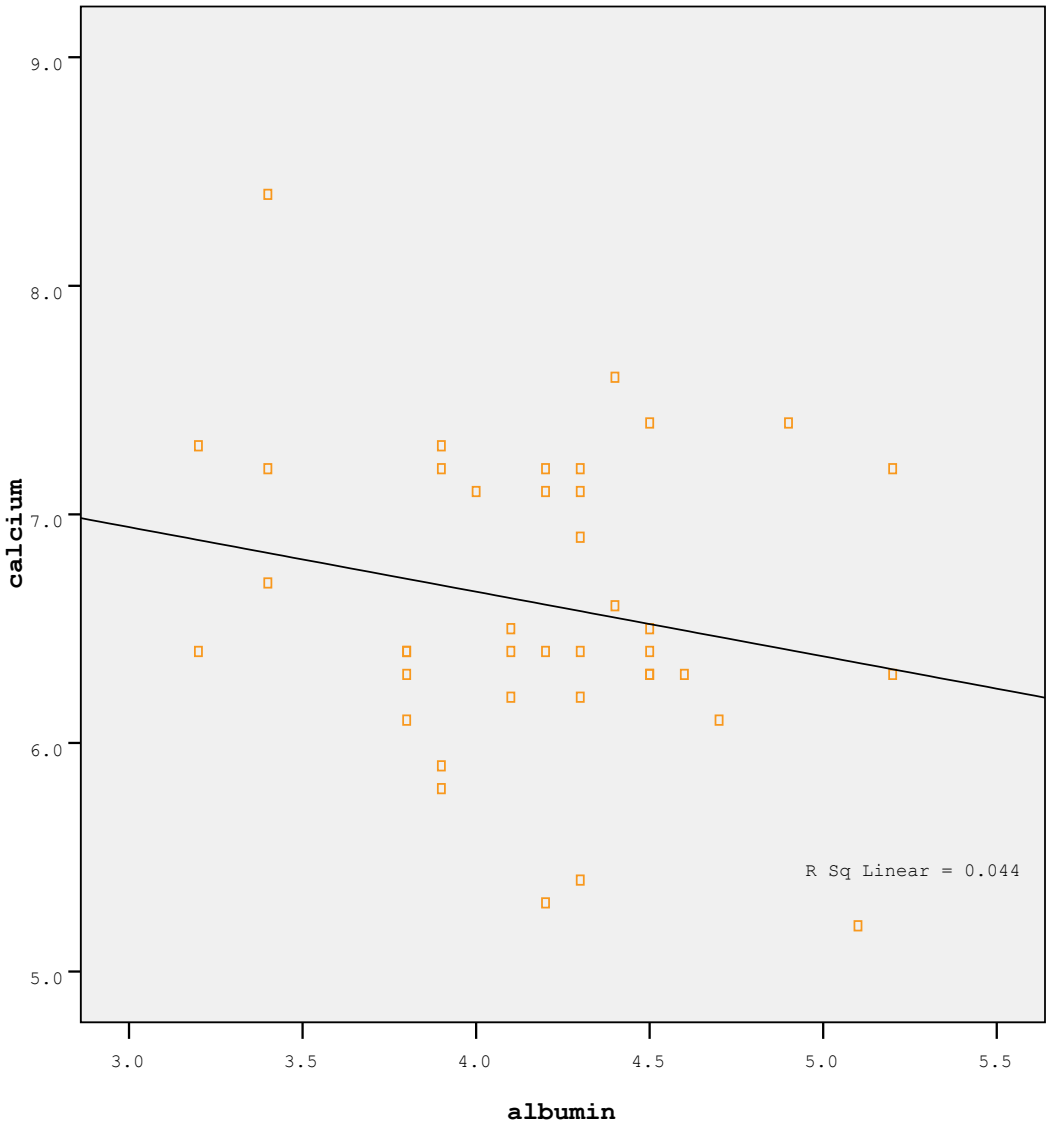

Figure (4-4): Show correlation between calcium and albumin (r =0.321, p.value 0.044).

Discussion

Polycystic ovarian syndrome is associated with increased ovarian and adrenal androgen secretions and hyperandrogenism and resultant hirsutism, hyperinsulinemia and central obesity. Although vitamin D primarily plays a role in bone metabolism, it has important functions in the reproductive system. Vitamin D receptors are found in ovarian and endometrial tissues and play an important role in steroidogenesis. Vitamin D deficiency is more frequently encountered in patients with polycystic ovarian syndrome (Stumpf 1989).

The main objective of this study was to assess the possible changes in serum calcium, AMH, albumin and estradiol levels among female patients with polycystic ovarian syndrome compared to healthy individuals in attempt to determine the effect of polycystic ovarian syndrome on these parameters.

In our study, we found that there was highly elevation in the concentration of AMH in patients sample when compared with control group and the difference was significant (P.value 0.000), also the highly reduction in concentration of serum calcium was found and the difference was significant also (P.value 0.000), Several studies showed that the level of AMH in plasma was significantly increased in patients with polycystic ovarian syndrome but there was significant decrease level of calcium among them. These findings were similar with that obtained by (Al-Hakeem 2009). The increased level of AMH was explained by the fact that AMH concentrations directly reflecting the increased number of early antral follicles. Moreover, the magnitude of AMH elevations in PCOS is associated with the extent of disease (Laven et al, 2004).

The reduced level of calcium was explained by the fact that Females with polycystic ovarian syndrome have vitamin D deficiency, 83% of all PCOS patients showed vitamin D deficiency while 35% were severely deficient (Sirmans 2013).Another study showed the opposite observations in which they suggest that abnormalities in calcium homeostasis may be responsible, in part, for the arrested follicular development in women with PCOS and may contribute to the pathogenesis of PCOS (Firouzabadi 2012).

Also it showed that there were no significantly difference between serum albumin and estradiol levels among female patients with polycystic ovarian syndrome compared to healthy individuals (P.value0.283) and (P.value0.086) respectively. The possible explanation of the decreased level of estradiol is the evidence that decreased aromatase activity may be a possible mechanism underlying the arrested follicular growth in PCOS. This was suggested by the study which showed that follicles in women with PCOS contain low levels of estradiol, aromatase mRNA and aromatase activity. PCOS follicular fluid contains one or more endogenous inhibitors of aromatase activity. 5α-androstane-3, 17-dione, a 5α- reduced androgen, is a competitive inhibitor of aromatase activity, it is markedly elevated in PCOS follicular fluid (Horng 2008). In addition, 5α-reductase activity is substantially higher in PCOS follicles than in control follicles, leading to increased production of 5α- androstane-3, 17-dione in women with PCOS. Collectively, the decreased estradiol production and increased androgen production in PCOS may be a result of elevated 5α-reductase activity and decreased aromatase activity (Horng 2008).In our study, we found that serum calcium did not show any significant correlation when compared with estradiol in PCOS patients, also did not show any significant correlation between AMH and estradiol. And it showed that there was insignificant inverse correlation between calcium and AMH and strong correlation between calcium and albumin, which may be due to significant increase of AMH and on the other hand the significant reduction on calcium level and the strong binding of albumin with calcium respectively.

Conclusion:

From the results and finding of this study, it is concluded that polycystic ovarian syndrome patients have decreased level of calcium and estradiol as well as albumin also decreased which may increase the risk of cardiovascular disease, osteoporosis and delay the course of treatment and recover of ovulatory problems.

Recommendation:

1. Using of calcium as indicator of increase the risk of cardiovascular disease, osteoporosis in patient with polycystic ovary syndrome.
2. Measurement and monitoring the concentration of calciotropic parameters on patients with polycystic ovary syndrome.
3. More studies should be carried out to clarify the effect of polycystic ovary syndrome in the calcium metabolism.
4. More studies should be carried out to clarify the effect of calcium in the treatment and recover of ovulatory problems.

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